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A VIROLOGICAL, CLINICAL AND EPIDEMIOLOGICAL STUDY OF COXSACKIE VIRUS INFECTIONS

By

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TORSTEN JOHNSSON
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This survey is based on the following communications

I. Gabinus, O., Gard, S., Johnsson, T. and A. Pöldre: *Studies on the Etiology of Epidemic Pleurodynia (Bornholm Disease)*. Clinical and Virological Observations. Arch. Virusforsch. *5*: 1 (1952).

II. Johnsson, T., and J. Lindahl: *Herpangina*. A Clinical and Virological Study. Arch. Virusforsch. *5*: 96 (1953).

III. Johnsson, T.: *Laboratory Infections with Coxsackie Viruses*. Arch. Virusforsch. *5*: 250 (1953).

IV. Johnsson, T.: *Studies on the Etiology of Bornholm Disease (Epidemic Pleurodynia)*. Epidemiological Observations. Arch. Virusforsch. *5*: 401 (1954).

V. Johnsson, T.: *Family Infections by Coxsackie Viruses*. Arch. Virusforsch. *5*: 384 (1954).

VI. Johnsson, T.: *Occurrence of Coxsackie Virus Infections in an Epidemic of Poliomyelitis*. Arch. Virusforsch. Vol. 6 (1955). In press.

VII. Johnsson, T.: *A Non-Typable Cytopathic Agent as the Probable Cause of a Family Outbreak of Aseptic Meningitis*. Arch. Virusforsch. Vol. 6 (1955). In press.

VIII. Johnsson, T.: *Simultaneous Recovery of two or more Immunological Types of Coxsackie Virus from the same Patient*. Arch. Virusforsch. Vol. 6 (1955). In press.

IX. Johnsson, T.: *The Occurrence of Coxsackie Viruses in a Hospital Material of Paralytic Poliomyelitis, Aseptic Meningitis and a Miscellaneous Group of Diseases*. Arch. Virusforsch. Vol. 6 (1955). In press.

X. Johnsson, T.: *A Histopathologic Study of Coxsackie Virus Infections in Mice*. Arch. Virusforsch. Vol. 6 (1955). In press.

XI. Johnsson, T.: *A Virological and Clinical Study of Infections with Coxsackie Virus*. In press.

In the following, references to these papers will be made in Roman figures. Arabic figures refer to the general reference list.

Translated by Kajsa and John Hogg.

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A virological, clinical and epidemiological study of Coxsackie virus infections

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TORSTEN JOHNSSON

A brief review of earlier investigations on aseptic meningitis and Bornholm disease in Sweden

Aseptic meningitis and Bornholm disease are both clinical conceptions of Scandinavian origin that have won recognition in the literature dealing with these diseases. The term acute aseptic meningitis was introduced in 1925 by Wallgren[1] to denote a benign disease of acute onset with fever, headache, stiff neck and pleocytosis, predominantly mononuclear. The term Bornholm disease (Bamble disease, epidemic pleurodynia or epidemic myalgia) derives from the Danish island of Bornholm in the Baltic where an epidemic took place in the summer of 1930, described by Sylvest[2] in his great, now classical, monography.

Considerable interest has centered around the etiology of the syndrome aseptic meningitis since the middle twenties. In 1926 Wallgren[3] published his first work on "méningitis ourliennes sans oreillons", which has later been described by a number of other authors. In a series of 76 "selected" cases of aseptic meningitis Alm[4] showed in six patients a diagnostic rise in specific mumps antibodies during convalescence.

The discovery in 1934 of the virus of lymphocytic choriomeningitis by Armstrong and Lillie[5] gave rise to the suggestion that this virus might be the cause of the aseptic meningitis that was so common in Sweden. Gard[6] and, later on, Alm[4] have devoted considerable attention to this question, but not in one single case — apart from a laboratory infection — did they isolate or find support for the occurrence of LCM virus in Sweden by serological studies.

From the studies by Malmgren[7] in 1941 it is known that Weil's disease is not uncommon in Sweden and that at least 50 per cent run an anicteric course. In his material there were 17 cases of leptospiral meningitis of which 5 were anicteric. His work, however, provides no answer to the question of the incidence of aseptic meningitis caused by Leptospira icterohaemorrhagiae.

Alm[4] considered he had found evidence of a primary herpetic infection of the C. N. S. Two of these herpetic infections ran a course typical of acute aseptic meningitis. He also performed a limited number of tests as regards the athropodborne encephalitides with negative result; this group of diseases is so far not known in Sweden.

In spite of intense research in laboratories and clinics over a period of several decades, the cause of aseptic meningitis in Sweden has only been elucidated to a very limited extent. When aseptic meningitis or non-paralytic poliomyelitis occurs in conjunction with paralytic poliomyelitis, the etiology has been assumed to be poliomyelitis virus. The incidence of aseptic meningitis, however, has varied in different epidemics without any satisfactory explanation being forthcoming.

Bornholm disease appears to have been widely spread in Sweden about a year after the 1930 epidemic in Bornholm; Huss[8] estimated the number of cases in Sweden in 1931 as at least 12,000. Minor epidemics occurred in the following years, one of which was described by Lindberg[9] in the fall of 1934, with emphasis on the clinical aspect. He seems to be the first to have established the occurrence of aseptic meningitis in five children with myalgia or pleurodynia and suggested that the different syndromes might have a common cause. Since 1934 no epidemic of Bornholm disease was reported in Sweden until the one in Jönköping and Huskvarna which forms part of the survey to be presented by the author.

From this brief review it should be apparent that the etiology of the aseptic meningitis has only been elucidated in a very small degree. One of the objects of this investigation has been to illuminate the relation between aseptic meningitis and Bornholm disease on the one hand and Coxsackie viruses on the other.

The Coxsackie virus group

In 1948 Dalldorf and Sickles[10] reported the discovery of a new agent in the feces of two children with paralytic poliomyelitis. The agent was said

to be probably of virus nature, its most characteristic features being its pathogenicity to suckling mice and hamsters. Adult animals of the same species were resistant to infection. This definition still appears to be valid in the case of freshly isolated strains. For the sake of convenience Dalldorf called this group of viruses "Coxsackie" after a small town of that name in the State of New York where the infected children were living.

On the basis of the pathologic changes produced in mice Dalldorf[11] divided Coxsackie viruses into two subgroups: subgroup A characterized solely by generalized destruction of striated muscles, and subgroup B characterized by focal areas of myositis, encephalopathy and fat necrosis.

The differences in histopathology are reflected in the clinical course of the disease produced in mice. A-strains give flaccid paralysis of skeletal muscles. They are often highly virulent with a fatality rate close to 100 per cent. B-strains, in primary isolation, are often much less virulent, display a masked neurotropism and produce symptoms characteristic of the involvement of the C.N.S-ataxia, tremor and spastic paralysis.

At present at least 12 immunologically distinct A types and 5 B types are known.[12, 13]

Virological investigation

Clinical material

In June 1949 a systematic study was commenced at the Department of Virus Research of Karolinska Institutet on the presence of Coxsackie virus in paralytic poliomyelitis and aseptic meningitis as well as other diseases of more or less unknown etiology. When it later became clear that myalgic symptoms, pleurodynia and herpangina might be related to Coxsackie virus, attention was also directed to patients with these symptoms. Cooperation was established in particular with the Stockholm Hospital for Infectious Diseases, where around 70 per cent of all cases admitted for aseptic meningitis and paralytic poliomyelitis were examined during a 4 years' period (7.1 1949 to 7.1 1953) (IX). Sporadic examinations have been made of samples from patients at other hospitals, as also from patients at the above-mentioned hospital after June 1953. At two hospitals for infectious diseases where Coxsackie virus appeared in epidemic proportions, more extensive investigations and field studies were carried out (Jönköping 1950 and Malmö 1952) (I, IV, VI). In addition, and partly in conjunction with the studies in Malmö, a study was made of the household

contacts of aseptic meningitis cases (V). The author hoped in this way to obtain an increased knowledge of the importance of Coxsackie virus as a causal agent in human disease, as also of possible variations in the clinical manifestations. The work in its entirety was completed on 7.1 1954 and thus extends over a period of 5 years; 3,000 patients — of whom about four-fifths hospitalized — were examined.

Specimens and methods

Specimens selected for examination comprised stools, throat swabs and spinal fluids, which were collected as early as possible, usually the day after admission and within two weeks after onset. In addition, acute and convalescent blood samples were as a rule drawn. During the first phase of the study infectivity tests were regularly performed on suckling mice with all three types of specimens. If virus was isolated from any of the specimens from a patient, stools practically never failed to yield virus. Therefore, in the later stages of the investigation the stools were examined first and, if positive results were obtained, throat swabs and spinal fluids were examined thereafter.

The method of examination has been described in detail in an earlier paper (XI) on only a brief occount will be given here. Suspensions of the specimens were clarified by centrifugation and as a rule inoculated intracerebrally and subcutaneously into usually 1 to 2 days old mice. During the first phase of the investigation the strains after passages and titration, were compared by means of crosswise neutralization tests. Later on, when Dalldorf's 14 type strains (A1—A10, B1—B4) became available, tests were made against hyperimmune sera prepared against these type strains.

A number of inoculated mice, including at least one representative of every type, were examined histopathologically.

In his most recent paper the author stated the general criteria for isolation of a strain. Since they appear important to a judgement of the results, they are repeated in this survey. a) A strain producing typical symptoms in infant mice should be established in passages, b) should be non-pathogenic to adult mice, c) should provide neutralizing antibodies to the virus in the patient's serum, preferably with a significant rise in titer from acute to convalescent phase, i.e. of at least 0.65 log units. d) It was diserable that the virus should be recovered in repeated attempts.

Table I. *Distribution of different types of Coxsackie virus subgroup A in the total material.*

Type	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	AX	Total
Number isol. ...	2	9	1	22	7	4	6	2	5	16	2	76

Table II. *Distribution of different types of Coxsackie virus subgroup B in the total material.*

Type	B1	B2	B3	B4	Total
Number isol. ...		7	38	6	51

Results and discussion

Number and type distribution of strains. During the five-year period 127 strains were isolated from 119 individuals; when the same type was recovered from different specimens from the same patient, the strains were counted as one. All ten A types of Dalldorf were represented as well as three B types, viz. B2, B3 and B4. In addition two non-typable mutually identical A strains occurred (tables I and II). Four per cent of all examined individuals were positive. No overlapping reactions were observed with the method of immunological typing employed by the author.

Isolations from different specimens. From all positive cases except one Coxsackie virus was isolated from feces. The exception was a positive throat swab in a patient whose feces specimen was negative. Throat swabs were positive in 17 per cent of the total number of positive cases. This figure is comparatively low and below the figures indicated by Parrot and associates.[14] If, however, throat swabs are divided into those collected within four days of the onset and after that time, the positives in the first period amount to 28 per cent. The routine testing of throat swabs over a period of several years, moreover, must involve a varying accuracy in the collection of the specimens. In two members of the same family virus was isolated from feces, and in one of them from throat swabs as well, three and nine days respectively before the onset. On the other hand repeat isolations of virus were achieved from the throat 13 days and in the stools 1 ½ months after the onset of symptoms.

In one of the cases of pleocytosis the spinal fluid was positive (type B3) (I). That Coxsackie virus subgroup B may occur in spinal fluid has since been confirmed by Hummeler and associates[15] (type B2), as also by Gear and associates,[16] who isolated type B4 from spinal fluid in 9 out of 21 cases of pleocytosis.

Inoculation routes. At practically all laboratories only one route of inoculation, usually the subcutaneous, appears to be employed in isolation tests. The author has been able to show, however, after performing simultaneous inoculations on two groups, one intracerebrally and the other subcutaneously, that the A strains were much more often positive in the case of the latter route, whereas the opposite was true of the B strains. The difference was highly significant ($p<0.001$) and emphasizes the importance of using both routes of inoculation for diagnostic purposes. This is in agreement with the experience that the B-strains are more easily adapted to the intracerebral than the subcutaneous route.

The incubation time of the isolation was as a rule not more than 10 days in the case of subgroup A, while with subgroup B it could be as much as 14 days. This means that the mice used in isolation tests should be kept under observation for at least 14 days. The mean incubation time of the first affected in each group was 4 to 5 days for subgroup A and 6 to 7 days for subgroup B, irrespective of the route of inoculation.

The clinical picture of the inoculated mice seemed to agree with that indicated in the literature for subgroups A and B. On some occasions the same isolation gave rise to flaccid and spastic paralysis in the same or different mice. When this happened, it was as a rule a result of mixed infection. In the case of one strain (type B3) the clinical picture differed from that usually seen in subgroup B. With this strain a flaccid paralysis often occurred after inoculation, followed in a day or so by spastic paralysis. This observation could not be explained by a mixture of different strains.

Histopathological changes in inoculated mice showed a remarkably good agreement with Dalldorf's division into subgroups A and B (X). Differences were found only as regards certain minor details. An exception of this kind occurred in animals inoculated with the above-mentioned type B3, the destruction of muscles being very pronounced and more like the picture seen in mice inoculated with subgroup A. Our experience thus differs from that of Melnick,[12, 17, 18] who often asserted that the classification into subgroups A and B was unwarranted.

The simultaneous occurrence of two or more strains from the same or different specimens in the same individual was not altogether uncommon. The technique employed for the separation of strains is described in an earlier paper (VIII).

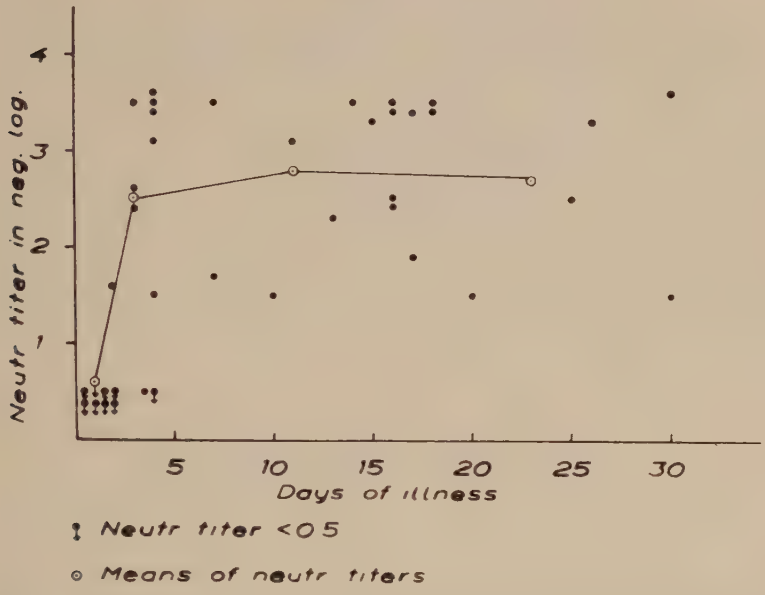

Fig. 1. Neutr. titer in relation to the numbers of days of illness. Subgroup A.

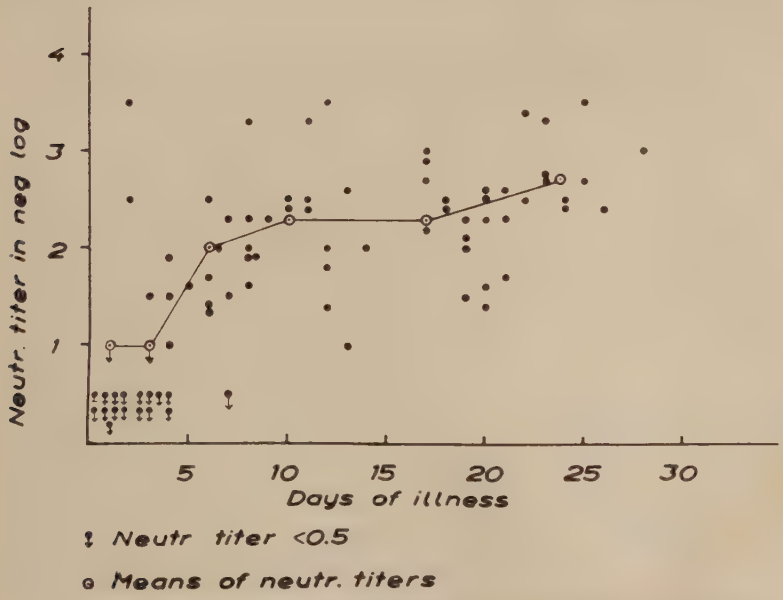

Fig. 2. Neutr. titer in relation to the numbers of days of illness. Subgroup B.

Figs. 1 and 2 demonstrate the occurrence of *neutralizing antibodies* in patient's sera in relation to the time of onset. In the case of subgroup A they appeared to attain their maximum during the first days after the onset, while in the case of subgroup B they rose more slowly and continued to rise slightly during the second and third weeks as well.

The criteria for a positive isolation were generally except in a few cases fulfilled. If antibodies were demonstrable in the patient's serum the case was included in the positive material. When antibodies were not demonstrable, as in a few cases of in-patients, the case was not included in the positive material and the finding was considered as a probable laboratory pick-up.

Clinical-epidemiological investigation

In this section reference is made to studies of 118 hospitalized patients in whom a virological or epidemiological connection with Coxsackie virus may exist (XI). Ninety-eight (29 subgroup A, 66 subgroup B and 3 subgroups A + B) of these cases may be denoted as "pure", exhibiting only such symptoms as are usually found in association with Coxsackie virus infections. A brief analysis of the clinical picture of these cases will be given. The association between different syndromes and Coxsackie virus will be illustrated, and epidemiological observations will be discussed.

Clinical picture and its relation to Coxsackie virus

It became evident in family studies in connection with the epidemic of Bornholm disease in Jönköping and Huskvarna in the fall of 1950, that the nosological entity of Bornholm disease could be classified under four syndromes, viz. aseptic meningitis, pleurodynia, myalgia and minor illness (I, IV). The not incommon occurrence of abnormal electroencephalograms points to an encephalitic component of the meningitic syndrom (XI). In the majority of cases, however, this component appears clinical masked and for this reason the term meningitis is used to cover also the varying degrees of cerebral involvement. The classification under these four syndromes, which were defined in an earlier paper (IV), has proved of great practical value to the subsequent study. In the same way it appears advisable to divide infections with Coxsackie virus subgroup A — as is done to a greater or

lesser-degree in the literature — into four syndromes, the most specific being herpangina. The others are aseptic meningitis or meningo-encephalitis, myalgia and minor illness.

The general signs and symptoms observed in the present series of Coxsackie virus infections, both subgroups A and B, did not differ from those described in the majority of the studies on Bornholm disease found in the literature. Of greater interest are the localized, more specific symptoms.

The small or minute ulcerative lesions of the posterior parts of the mouth and throat, first described by Zahorsky [19] and by him called herpangina, have been very rare in the author's material. Among 29 in-patients from whom subgroup A was recovered these symptoms occurred only in two. In the case of subgroup B they were observed in one patient. Similar efflorescences were also found in a laboratory infection where both subgroups A and B were isolated (III).

Pleocytosis (>4 cells per cu.mm) was found in 62 cases; the cell count did not as a rule exceed 400 and, in close to two-thirds of the cases, was between 11 and 100 per cu.mm. In those cases, where predominantly polynuclears were found, the observations were almost always referable to the first three days after the onset of symptoms. These circumstances accord with the normal experience of aseptic meningitis; the cell count was comparatively low, however.

In the cases in the literature where Coxsackie virus is stated to have been isolated from encephalitic patients, the symptomatology is very sparsely reported. Judging from the author's material it is evident that, in addition to pleocytosis, a mild affection of the central nervous system is a not uncommon phenomenon in Coxsackie virus infections. Thus the occurrence of convulsions and nystagmus in some cases, both in subgroups A and B, suggests a cerebral affection.

Signs of encephalitis were recorded in 9 out of 23 cases examined electroencephalographically — all nine except one had pleocytosis. Of particular importance is that, in six of the patients, the electroencephalographic record improved during the convalescence.

Severe encephalitic symptoms appear to be rather exceptional. One such case was a patient with herpangina and pronounced opisthotonos during the first days of illness with an after-course suggestive of a brain lesion.

The diagnosis was complicated, however, by a trauma of the skull a fortnight prior to the onset. Another patient with severe convulsions and nystagmus at the onset had abscences during convalescence. Abnormal electroencephalograms were recorded in both cases.

The convalescence one to two mounths after discharge was characterized in about 15 per cent of the cases by fatigue, irritability, muscle ache and headache. Pleocytosis was demonstrable in the majority of these cases.

In the symptoms discussed hitherto there has been no essential difference as regards subgroups A and B. The same applies to myalgia which occurred to an equal degree in both subgroups, whereas the extremely characteristic pleurodynia falls entirely under subgroup B. That these pains are typical of infections with B strains has been shown previously by the present as well as other authors. But claims have been mad in the literature that also A strains may produce pleurodynia.[20] In the author's comparatively extensive hospitalized material, however, these symptoms are entirely lacking in the subgroup A infections.

One further symptom is characteristic of subgroup B in the present material, viz. the recurrent course, especially the diphasic course of fever, which occurred in 50 per cent of the patients. Sylvest himself pointed out that this was typical of Bornholm disease.

Coxsackie virus subgroup A

The relationship borne by *herpangina* to certain A-strains (at least types 2, 4, 5, 6, 8 and 10) was convincingly demonstrated by Huebner and associates by,[14, 21—24] among other means, comprehensive epidemiological and statistical studies. The author's hospitalized material includes two sporadic cases of herpangina from which types A4 and A8 were isolated. Types A4 and A5 were found, moreover, in two out-patients. In this connections it may be mentioned that similar efflorescences were observed in a patient with aseptic meningitis and pleurodynia from whom type B3 was isolated. Since herpanginalike efflorescences were found in seven Coxsackie negative patients with other known virus infections, it is the author's opinion that similar lesions may occur unrelated to Coxsackie virus (II).

The isolation of Coxsackie virus subgroup A in cases of *aseptic meningitis* has in the literature usually been reported in connection with epidemics of

paralytic poliomyelitis or in sporadic cases (at least types A1, A2, A10 and other untyped strains) in which the epidemiological relationship has not been accounted for.[25—28 and others]

In the 354 examined cases of aseptic meningitis from the author's four-year material from the Stockholm Hospital for Infectious Diseases, Coxsackie virus subgroup A (types A1, A2, A4, A7, A10) was encountered in 2 per cent. All Coxsackie positive cases were children, and all except one occurred in the fall. If the percentage is counted on the number of children examined in the fall, the figure is 6.2 per cent (IX). On the entire material examined the corresponding figure is lower (4.2 per cent). Here all A types (A1—A10) but type A8 were represented. It should be pointed out, however, that this material includes cases from epidemics of aseptic meningitis in which a high percentage of Coxsackie virus subgroup B was isolated (XI).

Since in certain epidemics Coxsackie virus subgroup A has been recovered in a high proportion of the cases of paralytic poliomyelitis,[29, 30] it is very difficult to know what role Coxsackie or poliomyelitis virus plays in the individual case of non-paralytic poliomyelitis or aseptic meningitis. Only in exceptional cases have specimens from such patients been examined for the presence of polio virus as well. This was the case in an epidemic of paralytic and non-paralytic poliomyelitis in Easton, Pennsylvania, described by Melnick and associates.[29] In two Coxsackie positive cases of non-paralytic poliomyelitis attempts to isolate polio virus in monkeys were negative.

In connection the discussion on the encephalitic element in meningitis in the form of abnormality of the electroencephalogram the author was able very largely to exclude polio and mumps viruses as probable cause of these cases of aseptic meningitis or meningo-encephalitis on virological or serological grounds (XI). Some further cases of meningitis have been examined as regards polio. Together with the above-mentioned cases 22 patients have been tested. Seven cases lacked both neutralizing and complement-fixing antibodies against polio virus in serum collected during the convalescence, usually within a month of the onset; in one case the serum was collected 4 days after the onset. In one other case complement-fixing antibodies were lacking 14 days after the onset; no neutralization test was carried out. The remaining 8 cases examined exhibited neutralizing antibodies to one or more of the three virus types: complement-fixing antibodies were either lacking or existed in low titer, except in one patient who had

high titer (1/32), indicating a recent polio infection. Apart from the seven cases in which, on serological grounds, polio virus was judged to be an improbable cause of meningitis, negative results were obtained from inoculation in monkeys or tissue culture in two and four cases respectively. For epidemiological and serological reasons mumps virus was probably not responsible for the meningitis cases. The new group of "orphan viruses" discovered during the tissue culture period would appear to be more difficult to eliminate. In three cases, however, tissue culture yielded negative results.

Huebner and his associates[31, 32] maintained that the positive findings of Coxsackie virus among hospitalized patients was to a large degree due to nosocomial dissemination. This does not apply, however, to the author's material in which practically all stool specimens were collected a day or so after admission to the hospital when the patient was placed in an isolation unit. They also asserted that Coxsackie virus is so ubiquitous that it would be impossible to prove any connection between the virus and a particular syndrome other than statistically. It appears doubtful, however, whether our immunological conditions as regards Coxsackie virus are comparable to those under which the said authors work. The comparatively high percentage of positives in the miscellaneous group would indicate the ubiquitous nature of the virus. But this group contains a number of Coxsackie positive cases of herpangina, myalgia and minor illness, in which a relationship with Coxsackie virus subgroup A is generally considered to exist. Among the cases of paralytic poliomyelitis only a few A strains were recovered. With this group as control the author obtained a certain statistical support for a connection between Coxsackie subgroup A and aseptic meningitis.

Thus to some extent the author has been able to eliminate other conceivable causes of the Coxsackie positive cases of aseptic meningitis and statistically demonstrate a relationship with subgroup A. It is the author's opinion that such a connection does probably exist.

It may appear remarkable that in the Malmö epidemic, in which numerous A strains were recovered, none of them were related to a single case of aseptic meningitis. Subgroup A infections, however, often run so mild a course that the patients are never sent to hospital. Nor is pleocytosis altogether uncommon without outward signs of meningitis. In the material

reported on by Huebner and associates lumbar punctures appear to have been very seldom performed in cases of herpangina and minor illness.

Myalgia is usually not reported as a separate syndrome in the literature. In the author's material it often occurred in combination with aseptic meningitis. In several cases the lumbar puncture was negative and myalgia was the only diagnosis.

A number of epidemics of *minor illness* have been described in which one or more A strains were isolated.[33—38 and others] As a rule the studies were made in the field and lumbar punctures were not performed. There are a few of these cases in the author's material, of which one laboratory infection, the lumbar puncture being negative.

Coxsackie virus subgroup B

From a sporadic case resembling *pleurodynia* Curnen and associates[39] in 1949 reported the isolation of a Coxsackie virus (Conn. 5, type B1). On account of this observation Weller and associates[40] reinvestigated a material collected during an outbreak of Bornholm disease in Boston during 1949. They recovered Coxsackie virus (type B1) from the throat and observed an increase in neutralizing titer in paired sera in several cases.

In studying the possible occurrence of Coxsackie virus in aseptic meningitis Gabinus, Gard, Pöldre and the author (I, IV) — without knowledge of the above-mentioned reports — isolated Coxsackie virus from an apparently mixed epidemic of aseptic meningitis and epidemic pleurodynia. During this epidemic, Coxsackie virus type B3 was isolated from specimens from 7 patients, of whom three were diagnosed as pleurodynia. In some cases there was a significant rise of titer against type B3. While handling one of the strains from this epidemic three laboratory workers came down with the clinical diagnosis of pleurodynia (III). Type B3 was isolated from them and an increase in titer established. The demonstration by Shaw and associates[41, 42] that laboratory infections with type B1 produced pleurodynia-like illnesses provided additional evidence for the hypothesis that this agent might be responsible for the natural occurrence of Bornholm disease.

During the following years numerous papers were published, from many parts of the world, on epidemics of Bornholm disease with isolations of Coxsackie virus subgroup B, so that there can hardly be any doubt of the connection between this subgroup and the very typical syndrome of

pleurodynia. The isolated strains, however, have often not been immunologically typed. The relationship between this syndrome and types B1 and B3 appears to be well established. Patz *et al.*[45] in 1952 isolated type B4 from patients with Bornholm disease. Report of isolation of type B4 from a case of pleurodynia has recently been made by Wilkens *et al.*[54] In the author's material also B2 is represented in one case of pleurodynia. A typical epidemic of Bornholm disease probably caused by type B4 was observed by the author in the fall of 1954. A fifth B type has been isolated, but its clinical manifestations do not appear to have been published.[13]

Myalgia, as in the case of the A strains, appears to be reported in the literature rather as a symptom than as an independent syndrome. In the author's classification pleurodynia is not included under this conception, even if the borderline between the two may sometimes be indistinct. It is manifest that myalgia plays an important role as a clinical manifestation of infections by Coxsackie virus subgroup B. All three types recovered by the author are represented here. Two cases occur with normal spinal fluid.

Curnen and his coworkers[55] noted the prevalence of a *nonparalytic poliomyelitis*-like entity in Connecticut in 1948. A Coxsackie virus (Conn. 5, type B1) was isolated from the feces of 5 individuals with aseptic meningitis, and neutralizing antibodies against the agent were demonstrated in the serum of these patients and of 5 others. In the above-mentioned epidemic of Bornholm disease in Jönköping and Huskvarna the simultaneous occurrence of aseptic meningitis and pleurodynia or myalgia in the same or different individuals appears to have been observed for the first time since the discovery of Coxsackie virus (I, IV). Outwards signs of meningitis were often lacking, but lumbar puncture revealed pleocytosis. Coxsackie virus type B3 was recovered from cases of meningitis. The question arose whether the aseptic meningitis might have another cause than pleurodynia. No support was forthcoming for such an assumption, however. The appearance of a case in which Coxsackie virus type B3 was recovered, not only from the throat and stools but from the spinal fluid as well, provided important evidence of the etiological role of this type in aseptic meningitis. A laboratory infection with aseptic meningitis where Coxsackie virus type B3 was isolated appears to complete the chain of evidence (III). Later types B2 and B4 were also recovered from spinal fluid in cases of aseptic meningitis.[15, 16] Thus Gear and coworkers[16] isolated the latter type from spinal fluid in 9 out of 21 Coxsackie positive cases of aseptic meningitis.

In the author's four-year material from the Stockholm Hospital for Infectious Diseases Coxsackie virus subgroup B (types B2, B3 and B4) was isolated in 4.0 per cent of aseptic meningitis cases (5.8 per cent in children and 3.0 per cent adults) (IX). If this figure is compared with the corresponding figure of Coxsackie positives in the mixed control group, a significant difference is found ($p<0.01$). A similar comparison as regards the entire hospitalized material yields the same level of significance (XI). Furthermore in a number of Coxsackie positive cases poliomyelitis virus could be eliminated by serological tests, and in the Malmö epidemic all the stools from the Coxsackie positive cases of aseptic meningitis were tested on tissue culture with negative result. Such a series of arguments must be considered as satisfying high requirements in regard to the proof of a connection between an infectious agent and a disease.

Some aspects of differential diagnosis may be discussed in this connection. The biphasic course has come to be of some importance in the classification of aseptic meningitis as non-paralytic poliomyelitis. According to Fanconi[56] roughly one third of all cases of poliomyelitis run a temperature between 39° and 40° during the initial phase. Horstman,[57] on the other hand, pronounced a fairly low temperature to be typical of the initial phase. Recurrent fever appears to be usual in Bornholm disease, as in fact generally in infections with Coxsackie virus subgroup B. In the epidemic in Malmö a double-peaked fever curve occurred in six of the eight cases of aseptic meningitis in which a connection with Coxsackie virus type B3 was proven (VI). One of the temperature curves demonstrated in one of the figures in the paper dealing with this epidemic does not seem to deviate from the typical dromedary curve. Even if, in infections by Coxsackie virus type B3, the first phase exhibits a fairly high peak of fever more often than in poliomyelitis, it is obviously impossible to judge from the temperature curve whether a case of aseptic meningitis should be diagnosed as non-paralytic poliomyelitis, even though such a diagnosis would be suggested by the existence of a poliomyelitis epidemic. Such a biphasic course was also observed in the Malmö epidemic in a family infection of aseptic meningitis, probably caused by an "orphan virus" (VII).

Minor illness is seldom reported in the literature as an independent syndrome in connection with Coxsackie virus subgroup B. Types B2 and B3 occur in the author's hospitalized material with a significant rise of titer in connection with this syndrome.

Paralytic poliomyelitis and Coxsackie virus

The fact that Coxsackie virus was first recovered from two patients with paralytic poliomyelitis was to some extent the reason why special interest was devoted to the occurrence of this virus in polio and would explain why, in several American investigations of polio, Coxsackie virus subgroup A was found fairly often. Melnick and his associates[25] described an epidemic in which they recovered this agent in more than 50 per cent of the cases. In the author's material comprising 522 cases of paralytic poliomyelitis Coxsackie virus was isolated in a few cases, amounting to about 1 per cent (XI). In all the positive cases exept one the virus belonged to subgroup A. Both mild and severe cases are represented. The one B strain was type B3. Subgroup B does not appear to have been found earlier in cases of paralytic poliomyelitis. Since Coxsackie virus subgroup B and polio virus have only once previously been demonstrated in the same specimen,[58] it would have been of interest to attempt the isolation of polio virus in this case. The specimen originates, however, from the earlier phase of this work and had been used up by the time we started with tissue culture.

Some diseases of special interest

It would have been expected that a disease in which the myalgic symptoms are so pronounced as in *polyradiculitis cervicalis infectiosa* might be caused by Coxsackie virus. All 35 cases examined, however, were negative. It is stated in the literature that Coxsackie virus has been recovered in individual cases of *Guillain-Barré's syndrome*.[59,60,61,62] Every one of 16 cases examined with this syndrome and 8 cases of *polyneuritis* in the author's material were Coxsackie negative.

Family infections

It was the familial occurrence of Coxsackie virus and the varying clinical manifestations in the epidemic in Jönköping and Huskvarna which to some extent gave rise to the more systematic study of the spread of Coxsackie virus infections in the family (I, IV). Since at the same time the question of the etiological importance of Coxsackie virus in aseptic meningitis was one of the main points of interest, the latter syndrome was chosen as point of departure.

In 19 out of 21 family members examined (90 per cent) serological or virological evidence existed of an infection with type B3, suggestive of a widespread dissemination within the families (V). The diverse symptoms were representative of different clinical manifestations of one and the same infection. In one family all four syndromes were present. Inapparent infections were rare; 91 per cent were taken ill.

As regards subgroup A the number of Coxsackie positive families was too small, and a simultaneous polio infection in one family complicated the assessment of the role of Coxsackie virus. In one family with type A2 aseptic meningitis as well as myalgia and minor illness were represented. In the aseptic meningitis case and in one case of minor illness in the same family neutralizing antibodies were lacking to all polio virus types.

There was a statistically significant difference ($p<0.01$) between adults and children concerning the frequency of myalgic symptoms and pleurodynia. The same statistical significance was found in 21 family outbreaks in Jönköping and Huskvarna. It was already apparent in the latter epidemic that aseptic meningitis was more common (80 per cent) in children than in adults as demonstrated by lumbar punctures. It is also remarkable that in respect to subgroup A in the author's four-year material from the Stockholm Hospital for Infectious Diseases all cases of aseptic meningitis were children.

It thus appears likely that in infections with type B3 myalgia and pleurodynia are more common in adults than in children whereas aseptic meningitis is the more common manifestation of the infection in children. These observations are in some degree confirmed by Gear and coworkers [54] who in an epidemic of Bornholm disease found pleurodynia to be more common in adults than in children. Thus the character of an epidemic of Coxsackie virus type B3 infections may be influenced by the preponderance of the different age groups. In a community without previous experience of infections a large proportion of the patients will be adults, the epidemic will be considered largely one of pleurodynia. On the other hand, in places where a considerable proportion of the adult population is immune from previous exposure, an epidemic will involve mainly children and the symptoms will generally be milder; myalgia and pleurodynia will not be a prominent feature.

The study of the family outbreaks provides some idea of how Coxsackie virus spreads within the family. In the six families with more than one

member affected the first patient was in every case a child, and only one had attained school age. An interval of four days usually followed, indicating that case no. 2 in each family was secondary to the first. In this investigation it appears that the infection came to the family through children in the pre-school age. The incubation period appeared to be on an average five days. Since virus has been isolated nine days prior to the onset, it appears probable that the incubation period might sometimes be longer.

Some epidemiological observations

Monthly distribution. Nearly all isolations occurred during the second half-year periods with maximum in September, as shown in fig. 3. The distributions of A and B strains were practically equal.

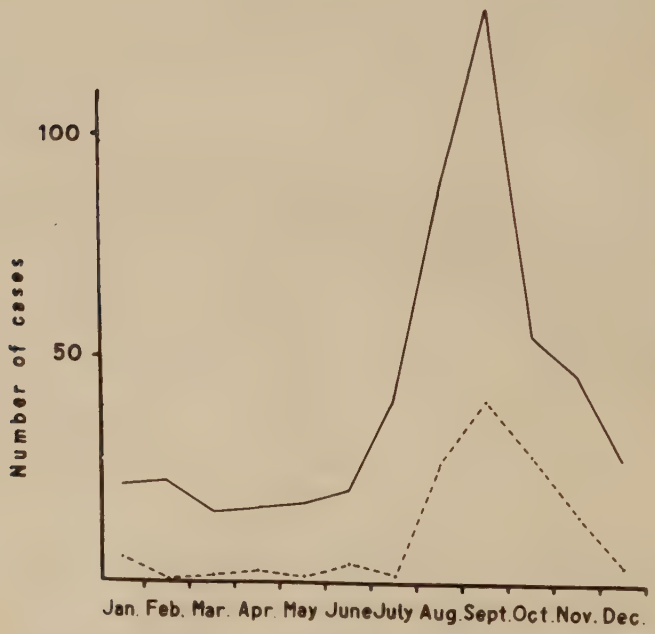

Fig. 3. Monthly incidence of cases of aseptic meningitis (uninterrupted line) admitted to the Stockholm Hospital for Infectious Diseases and 4 children's hospitals in Stockholm during 1949—1952, and monthly incidence of total number of isolated Coxsackie virus strains (dotted line) during 6.1.1949—7.1.1954.

Yearly variation. During the five-year period of the investigation no distinct regularity in the appearance of the immunological types was noticeable. The most common B type, B3, after reaching a maximum in 1952 was, however, entirely lacking in the following year. The same applied, with one exception, to types A4 and A10. In 1953 solitary strains of types B4 and A7 were isolated; in the following year these two strains completely dominated the Coxsackie flora; among some 90 strains isolated and 58 so far typed in that year type B3 was not represented.

Epidemiological relation between Coxsackie virus, aseptic meningitis and paralytic poliomyelitis. The recovery rate of Coxsackie virus (subgroups A and B) from cases of aseptic meningitis in the author's four-year material from Stockholm was practically the same during spring and fall periods (7.1 and 5.6 per cent respectively) (IX). As a comparison may be mentioned that during the spring periods, when mumps has a distinct peak, two cases of mumps meningitis without parotitis were diagnosed by complement fixation test, while no such cases were found during the fall periods. In terms of actual figures the number of Coxsackie virus infections in the author's entire material was greatest in September, which is the peak period for aseptic meningitis. During 1949—1950 this peak of aseptic meningitis in Sep-

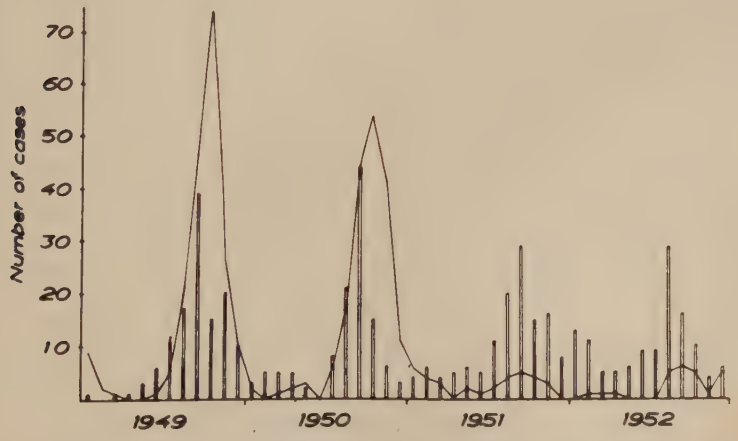

Fig. 4. Monthly incidence of cases of paralytic poliomyelitis (curve) and aseptic meningitis or non-paralytic poliomyelitis (columns) admitted to the Stockholm Hospital for Infectious Diseases and 4 children's hospitals in Stockholm during 1949—1952.

tember occurred one month before that of paralytic polio, indicating a different etiology (fig. 4). A different etiology was likewise established in the Malmö epidemic where type B3 was the probable cause of a considerable part of the cases of aseptic meningitis during the first month of the epidemic.

Lindberg, in his description of the epidemic of Bornholm disease in the fall of 1934, pointed out that paralytic poliomyelitis had not occurred in the epidemic area. The same observation was made in the Jönköping and Huskvarna epidemic. Dalldorf,[63] on the strength of experimental and epidemiological studies, advocated the opinion that an antagonism exists between Coxsackie and polio viruses. In connection with his investigations of interference phenomena Dalldorf pointed out that, during the great epidemic of Bornholm disease in Sweden in 1931, comprising 12,000 reported cases, the number of paralytic and lethal cases of poliomyelitis was the lowest observed over a 20-year period. The difference between the observed and the expected incidence of poliomyelitis was supported by statistics.

From 1950 to 1952 type B3 seems to have been widespread all over the world (U.S.A.,[31, 49] England,[64] Sweden (I, IV, V, VI), Australia[65] etc.). Among the reports on these epidemics of type B3, "no cases of paralytic poliomyelitis appeared in this region" was a very frequent comment. In the author's material there is, however, a manifest exception. During a 14-day period within the first phase of the Malmö epidemic Coxsackie virus type B3 was recovered from a number of cases within a limited area in which several cases of paralytic poliomyelitis appeared simultaneously. Coxsackie and polio viruses, however, were never isolated from the same specimen or in the same family (V, VI). In the fall of 1950, when paralytic poliomyelitis was prevalent in Stockholm, types B2 and B4 were both recovered. The question of interference between these two agents must be further studied experimentally and epidemiologically.

General discussion

The occurrence of the numerous A strains in the field studies of the Malmö epidemic, with its small number of Coxsackie positive hospitalized cases, suggests that infections by A strains usually run a milder course than

B strains. Severe clinical manifestations may, however, possibly occur in certain subgroup A infections. It is also probable that this subgroup may give rise to aseptic meningitis. As regards subgroup B there is no doubt of this.

In the author's four-year material Coxsackie virus was recovered from, on an average, 6 per cent of all cases of aseptic meningitis. In the distribution of Coxsackie positive cases of aseptic meningitis among children and adults, and subgroups A and B, certain variations come to light. Barely 12 per cent of the children were positive and 3 per cent of the adults. As regards subgroup A all positive cases were children (barely 6 per cent), and as regards subgroup B barely 6 per cent were children and 3 per cent adults. If account is taken of the fact that, according to our experiences from the epidemic in Jönköping and Huskvarna and the family studies, the recovery rate of type B3 is about 50 per cent in children and 25 per cent in adults in otherwise established acute Coxsackie virus infections, the following assumption may be made. About 12 per cent of the aseptic meningitis cases in the above-mentioned material — if the other B types behave in the same way as type B3 — were caused by Coxsackie virus subgroup B. If this observation is extended to the A strains as well, the corresponding figure would be 4 per cent. This would mean that Coxsackie virus was responsible for about 16 per cent of all cases of aseptic meningitis in the present material. It is probable that, during 1949 and 1950, a large proportion of the remainder were due to polio virus. But during 1951 and 1952 the incidence of paralytic poliomyelitis was very low. It has been claimed that the incidence of Coxsackie virus in such years is comparatively high in contrast to years when paralytic poliomyelitis is widespread. In the author's material, however, there is no such difference in the incidence of Coxsackie positive cases of aseptic meningitis during the two above-mentioned periods. In the fall of 1953 there was a severe epidemic of paralytic poliomyelitis in Stockholm. The following year there was again a high incidence of aseptic meningitis. Now, however, tissue culture was available and Svedmyr[66] isolated "orphan viruses" in about 35 per cent of the cases of aseptic meningitis in Stockholm. As the serological typing of these strains was not completed at the time of writing it cannot be excluded that some of these orphan strains are in fact Coxsackie viruses.

It is thus probable that the aseptic meningitis referred to in the introduction and described by Wallgren[1] in 1925 was to certain extent attributable

to Coxsackie virus. There is reason to presume that part of the responsibility was also borne by non-typable cytopathogenic agents, orphan viruses. Since, moreover, polio virus must be considered as an etiological factor, the responsibility for the majority of aseptic meningitis cases be would borne by these agents in conjunction. This was as a matter of fact also true of the epidemic of aseptic meningitis and paralytic poliomyelitis in Malmö.

The hypothesis advanced by Lindberg[9] of a common cause of Bornholm disease and the aseptic meningitis associated with it has been brought into the realm of high probability by this study of Coxsackie virus infections.

Summary and Conclusion

1. During a period of about five years 6.1 1949 to 7.1 1954, the author investigated about 3,000 persons for the occurrence of Coxsackie virus, chiefly consisting of hospitalized patients, of whom half had either the diagnosis of aseptic meningitis or paralytic poliomyelitis. A small number derived from epidemiological studies in the field including family infections.

2. Isolations were made of 127 strains from 119 individuals — in some cases two or more strains occurred simultaneously in the same individual. Seventy-six were A strains and 51 B strains. Types A1 to A10 and two mutually identical nontypable strains were represented among the former, and types B2, B3 and B4 among the latter.

3. Histopathological changes in inoculated mice showed very good correlation to Dalldorf's division into subgroups A and B.

4. In isolation tests involving the simultaneous inoculation of one group intracerebrally and one group subcutaneously the A strains were more often positive in the case of the latter route, whereas the opposite was true of the B strains.

5. Among the feces positive cases throat swabs collected prior to the fifth day of illness were more often than those taken subsequent to the fourth day of illness. In two cases Coxsackie virus was isolated 3 and 9 days respectively before the onset. In one case in which virus was found in feces and throat swab, spinal fluid was also positive.

6. In the case of Coxsackie virus subgroup A the neutralizing antibodies in the patient's serum attained their maximum during the first days after the

onset, while in the case of subgroup B, they rose more slowly and continued to rise slightly during the second and third weeks as well.

7. At an early stage of the investigation the author was able to establish by means of virological and serological studies that type B3 was in all probability the cause of the epidemic of Bornholm disease; type B3 was isolated in 7 out of 20 cases. Aseptic meningitis was common in this epidemic and was probably caused by the same agent, which was confirmed in one case by its appearance in spinal fluid. There was an obvious familial occurrence of Bornholm disease.

8. With hospitalized cases of aseptic meningitis as a point of departure the author conducted a systematic study of the familial occurrence of Coxsackie virus infections. In three families Coxsackie virus subgroup A and in five families subgroup B (type B3) was recovered from 2 to 3 members of the same family. As regards subgroup B all clinical manifestations of Bornholm disease by the author classified as four syndromes viz. aseptic meningitis, pleurodynia, myalgia and minor illness, were represented. Coxsackie virus type B3 and the corresponding antibodies were demonstrated in a great majority of the family members who, with a few exceptions, had been ill during the time in question. Aseptic meningitis was more common in children. Pleurodynia and myalgia, on the other hand, were more common in adults. It appeared likely that the infection was introduced into a household through one of the children, from whom it spread to the other members.

9. The less specific symptoms from infections both with A and B strains resembled those described in connection with Bornholm disease and herpangina. Stitch and recurrence of fever were found in about half of all cases infected with subgroup B, but were lacking in subroup A. In two cases there were herpangina-like efflorescenses in connection with subgroup A, and in another case this syndrome was combined with aseptic meningitis and pleurodynia in connection with subgroup B. Coxsackie positive cases of aseptic meningitis were usually mild and not seldom lacked objective signs of meningitis apart from pleocytosis. On the other hand, there were sometimes signs of encephalitis — usually of a mild character. In eight out of 23 examined cases of aseptic meningitis or meningoencephalitis there were abnormal electroencephalograms.

10. In a four-year material of hospitalized cases, some 70 per cent of which were examined, Coxsackie virus was isolated in about 6 per cent (not quite 12 per cent children and 3 per cent adults). It was probable that a connection existed between Coxsackie virus and aseptic meningitis; this was particularly manifest as regards subgroup B. It was presumed that Coxsackie virus, polio virus and "orphan virus" are responsible for the majority of cases of aseptic meningitis.

11. In 522 cases of paralytic poliomyelitis, Coxsackie virus subgroup A was isolated in 1 per cent and subgroup B occurred in one patient.

12. In a mixed epidemic of Coxsackie virus subgroup B and paralytic poliomyelitis a considerable part of the aseptic meningitis cases at the beginning of the epidemic were probably caused by type B3. This type appeared simultaneously with paralytic poliomyelitis within the same block of buildings, but was never found in conjunction with cases of paralytic poliomyelitis.

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In the spring of 1949 Professor Sven Gard initiated the study of Coxsackie virus in his laboratory, especially in regard to its relation to aseptic meningitis. I had the opportunity of continuing this work under his excellent leadership. For this privilege I am profoundly indebted.

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